PCB Dissipation and Microbial Community Analysis in Rhizosphere Soil Under Substrate Amendment Conditions

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ABSTRACT

Dissipations of PCB in soils under factorial combinations of soil amendment (biphenyl, pine needles, orange peels, unamended) and planting (reed canarygrass, flat pea, burr medic, unplanted) regimes were evaluated in relation to carbon substrate utilization patterns of corresponding soil microbial populations. We examined potential differences among microbial communities under the treatments and then related the differences to PCB dissipation. Based on univariate analysis of treatment results, three PCB dissipation groups were identified. They were (1) high: 50% or more loss of an original 50 mg/kg aroclor 1248 after 100 d, (2) medium: 40 to 50% loss, and (3) low: <20% loss. A canonical discriminant analysis (CDA) was performed on the data with the PCB dissipation grouping as the class variables and the carbon substrates as exploratory variables so as to relate PCB dissipation to C substrate use. The three PCB dissipation groups were reconstructed based on a subset of C substrates. The CDA identified C substrates that were most important in differentiating between population communities under the various treatments. The potential importance of the ability to correlate community substrate utilization to contaminant dissipation is discussed.

KEY WORDS: PCB dissipation, carbon utilization patterns, canonical discriminant analysis.

I. INTRODUCTION

The knowledge that plant root zones (rhizospheres) provide unique environments for the enhanced degradation of organic chemicals has generated renewed interest to capitalize on the processes involved in order to develop remediation strategies for
xenobiotic organic contaminants. Accelerated dissipation of agricultural chemicals from vegetated soil systems has been reported since the late 1970s (Hsu and Bartha, 1979; Reddy and Sethunathan, 1982; Sandmann and Loos, 1984). However, concerted efforts to take advantage of plant systems for remediating organic contaminants did not gain momentum until recently. Within the last 5 years, plant species from a wide range of families have been reported to enhance the dissipation of contaminants belonging to every major chemical group implicated in environmental contamination, including petroleum hydrocarbons (Schwab and Banks, 1997; Qui et al., 1997), various families of pesticides (Anderson et al., 1994; Alvey and Crowley, 1996; Hoagland et al., 1997; Burken and Schnoor, 1998), energetic nitroaromatic compounds (Thompson et al., 1997; Tobin, 1997), and chlorinated solvents (Walton and Anderson, 1990).

The major mechanisms involved in plant-mediated removal/destruction of organic contaminants are uptake, translocation, and metabolism collectively termed phytodegradation, or biodegradation mediated through root-microbe interactions, also known as rhizodegradation. Of the two processes, rhizodegradation appears more amenable to manipulations; uptake and translocation processes are modulated by plant anatomy and physiology, and lipophilicity of a chemical, both of which are less readily amenable to modification. A large body of evidence exists in the literature on modifications of plant rhizospheres in order to improve plant nutrition (Okon and Hader, 1987), or for protection from pathogenic species (Ramachandra-Reddy, 1959; Agnihotri, 1964; Balasubramanian and Rangaswani, 1973; Kloepper et al., 1991). In spite of the upsurge in research on plant-mediated bioremediation, the development of rhizosphere manipulation strategies for improving rhizodegradation for practical use in the field has been slow. A major obstacle has been a lack of adequate information about rhizosphere microbial communities and their specific interactions with plant systems to enhance the dissipation of particular contaminants. This, in turn, has largely been a result of limitations of the most commonly used microbial characterization methods in environmental matrices. Traditional isolation-based methods for characterizing microbial populations are severely limited by media selectivity and nonculturability of the majority of the population members (De Leij et al., 1993). While DNA-based method can provide information about taxonomic diversity within microbial communities (Torsvik et al., 1990a, b), they do not offer much information about specific interactions and how they determine functions such as biodegradation of organic contaminants in a matrix. Additionally, they are limited in their routine application by time, equipment, and cost considerations.

Over the last decade, the analysis of carbon utilization patterns within microbial communities has gained increased acceptance for characterizing functional diversities within those communities. Since it was first described by Garland and Mills (1991) as a means for describing ‘physiological profiles’ of microbial populations, C substrate utilization patterns have been used as a relatively simple yet definitive method for distinguishing between microbial communities in various ecosystems, including populations in soil from different desert plant systems (Zak et al., 1994), a model laboratory bacterial population (Haack et al., 1995), a hydroponics system (Garland 1995), and spermospheres (soil immediately surrounding plant seeds) in different soil types (Buyer et al., 1999). However, this approach has not been used
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to characterize rhizosphere populations in contaminated systems. The characterization of microbial communities involved in rhizodegradation is crucial to developing strategies in order to manipulate such populations to enhance their degradation capabilities for use in the field. Carbon substrate utilization patterns may offer an insight into functional characteristics of degrader communities in specific rhizospheres.

Recently, we reported experiments that investigated the use of soil amendments with or without planting to enhance PCB dissipation in soil (Dzantor and Woolston, 2001). The soil amendments were biphenyl, the synthetic structural analog to PCBs, or ground orange peels and pine needles, all of which have been reported to stimulate PCB dissipation in soil (Brunner et al., 1985; Hernandez et al., 1997). Two of the plant species selected for this study were reed canarygrass (Phalaris arundinacea L.), and the legume flat pea (Lathyrus sylvestris L.) that previously showed inherent potentials for stimulating PCB dissipation in greenhouse experiments (Dzantor et al., 2000). A third plant, burr medic (Medicago polymorpha), showed similar potentials during a preliminary (unpublished) growth chamber experiment. In this report we evaluate the PCB dissipation patterns in soil under different soil amendment and planting regimens and explore the feasibility of establishing potential correlations between PCB dissipation, C substrate utilization patterns, and utilization of specific substrates by microbial populations under the various treatments.

II. MATERIALS AND METHODS

A. Crops

The plant species selected for this study were reed canarygrass (Phalaris arundinacea L.), flat pea (Lathyrus sylvestris L.), and burr medic (Medicago polymorpha). Reed canarygrass and flat pea were among crops that showed inherent potentials for stimulating PCB dissipation during a previous greenhouse experiment (Dzantor et al., 2000). Burr medic is one of three medics under investigation for the potential enhancement of rhizodegradation. The biochemistry and molecular genetics of medics, which include alfalfa (Medicago sativa), are well characterized, so they may serve as model systems for studies that involve elucidating and manipulating rhizodegradation at the molecular level to improve currently observed limitations of selected plant species.

B. Soil and Soil Preparation

Hatboro silt loam (coarse-loamy mixed nonacid, mesic, Fluvaquent) was collected from the Central Maryland Research and Education Center, Clarksville Facility. It has the following characteristics: sand, 61%; silt, 22%; clay, 8%; OM, 3%; CEC, 5.72 cmol/kg; pH (1:1 water) 6.9. PCB contamination was simulated in the laboratory by spiking soil with stock solutions of aroclor 1248 (ChemService Inc., West Chester, PA; 98% purity) in hexane to give concentrations of 50 mg PCB/kg oven dry soil (ods). The spiked soils were mixed thoroughly by sieving several times and placed under a hood to allow the solvents to evaporate. Portions of spiked soil were mixed thoroughly with ground pine needles or orange peels to give final
amendment levels of 2% w/w in soil. Another portion of PCB-fortified soil was amended with biphenyl (1,1’-biphenyl, 99% purity, ChemService Inc., West Chester, PA) at a rate of 1000 mg/kg ods. All amended soils were sieved to pass 2–mm sieve. Portions of soil were left unamended to serve as controls for the treatment combinations. After treatment, soils were stored at 4°C for at least 2 weeks before use.

C. Plant Growth Units

Plants were grown in laboratory microcosms called Moisture Replacement Systems (MRS) that were developed by Sardanelli et al. (1995). The units consist of polyfoam-insulated boxes (62.5×30×20 cm) with top halves that hold cups for the rooting medium (soil) and bottom halves that hold approximately 9L of H2O. Each cup, a 50–ml conical tube in these experiments, has a wick that extends from the lower 1/3 of the tube into the lower 1/2 of the box. The system uses hydraulic transport via the wick from the enclosed water reservoir to the rooting medium. In addition to allowing less soil and water to be used than in standard pot testing, the MRS allows complete containment of contaminated soil, thereby eliminating spread of contamination.

D. Experimental Procedure

About 50 g of soil from the individual treatments were placed in the conical tubes held in the top half of the MRS units. Prior to seeding, the top halves were placed on the bottom water reservoirs and left to equilibrate overnight, after which the soils were seeded with the individual crop species. Once healthy stands were established, the plants were thinned to no more than two plants per cup. The experiments were performed in a factorial experimental design with four plant systems (unplanted, flat pea, burr medic, and reed canarygrass), and four soil amendment regimes (un-amended, orange peels, pine needles, and biphenyl) to yield 16 treatment combinations. Each treatment was replicated three times. The unplanted, unamended soils served as controls for plant and amendment effects, respectively.

After planting, the MRS units were placed on laboratory shelves that were fitted with lighting at a 12–h photoperiod. At harvest, soil and plants were removed from the cups. The shoots were separated from the contents of the cups, which were considered rhizosphere soil by virtue of the extent of root development in each cup. The contents were macerated using razor blades and mixed thoroughly. One portion of the soil was stored at 4°C for microbial analysis, and the other portion was stored in the freezer for chemical analysis.

E. Microbiological Analysis

Carbon utilization patterns of microbial communities from various treatments were analyzed by the BIOLOG technique using Biolog Eco Microplates (Ecoplates, Biolog Inc., Hayward CA). Each Ecoplate contains 96 microtiter wells with three replicates of 31 carbon sources representing carbohydrates, amino acids amines, carboxylic acids, polymers, and miscellaneous substrates (Zak et al., 1994). Three water wells served as controls. Using a Wheaton multipipetter (Wheaton Science Products), Ecoplates were inoculated with 100 µL of a 10⁻² dilution in a 10-fold
dilution series for individual replicates in each treatment. The inoculated plates were incubated at 30°C for 5 days and C utilization, as indicated by color intensity of the respiratory dye in each well, was read at 590 nm using a Maxiline Microplate Reader Emax (Molecular Devices Corp., Sunnyvale CA). In this soil, the incubation temperature of 30°C and incubation period of 5 days were found to provide the most meaningful comparative data on C-substrate utilizations in our treatments.

F. Chemical Analysis

PCBs were extracted from soil using a shake flask protocol described previously (Dzantor and Woolston, 2001). Briefly, 5–g (ods) portions of soil at 20 to 25% moisture were placed in a 125–ml Erlenmeyer flask and slurried with 1 ml H2O. Twenty milliliters of a 1:1 hexane acetone mixture were added to the soil and they were extracted on a rotary shaker at 180 rpm for 1 h. The solvent was decanted and the extraction procedure was repeated. Following the second extraction, the two solvent portions plus the soils were vacuum filtered through glass microfiber filters (Whatman GF/D) using Buchner funnels. The filtered supernatants were made to known volume, and aliquots were prepared for GC analysis by cleaning with concentrated sulfuric acid (1:1). Samples were analyzed on an HP 6890 GC with electron capture detection. The GC column was an HP 5–MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). The oven was temperature programmed from an initial 80˚C rising at a rate of 15˚C/min to 320˚C. Injector and detector temperatures were 250 and 300˚C, respectively. The carrier gas was He at constant flow rate of 2 ml/min and make-up gas was N2 at 60 ml/min. Freshly spiked soils (50 mg PCB/kg ods) were extracted and analyzed concurrently with samples to enable assessment of the extraction procedure at each period. Aroclor concentrations were quantified by comparing total area counts of 15 selected peaks in sample extracts with area counts of corresponding peaks (i.e., same retention times) in standards of aroclor 1248 that were run concurrently with samples and spiked analytical controls. PCB recoveries from the spiked controls averaged 98% of spike (sd =11; n =8) over the extraction period.

G. Statistical Analysis

Data were analyzed using the SAS system (The SAS Institute, Cary, NC). PCB dissipation data were analyzed using univariate ANOVA. For analysis of C utilization patterns, absorbance values of three replicates of each carbon substrate on a plate (96 wells total) were averaged and then corrected for plate variation by subtracting the average absorbance for the water wells. This reduced the total number of variables per plate to 31. The resultant data were examined using ANOVA to discern differences in carbon utilization patterns between the various treatments. In order to relate patterns of individual substrate utilization to PCB dissipation, the data were further analyzed using canonical discriminant analysis (CDA) in which individual carbon substrates were used as explanatory variables, and PCB dissipation patterns identified as high, medium, or low based on the ANOVA were used as the class variables. The objective was to identify linear functions that could differentiate
between PCB classes, and C substrates that are important in the construction of such functions.

III. RESULTS

After 100 d of incubation, nearly 70% of an initial 50 mg/kg addition of aroclor 1248 was recovered in soil that was unamended and unplanted. In soils that were unamended but planted, recoveries ranged from 65% of initial additions in flat pea rhizospheres to 55% in burr medic rhizospheres. For soils that were amended, but left unplanted, recoveries ranged from 59% when soil was amended with pine needles to 44% when soil was amended with orange peels. Combining soil amendment and planting produced little or no enhancement in PCB dissipation compared to dissipation with soils that were amended but not planted (Dzantor and Woolston, 2001).

ANOVA indicated a significant plant-by-amendment interaction (p<0.05), which was found to be due to inhibition of PCB dissipation in biphenyl-amended, burr medic planted soils. In this treatment > 80% of initial additions of PCB remained in soil after 100 d. Consequently, this treatment combination was excluded and ANOVA was performed again, which indicated no significant plant-by-amendment interaction. In summary, plants did not affect PCB dissipation significantly, whereas amendments reduced PCB content significantly (p<0.05) compared with unamended controls. Tukey mean comparison test allowed grouping of PCB dissipation patterns into the three classes shown in Table 1. In order to relate C substrate utilization and PCB degradation, the PCB dissipation classes in Table 1 were used as class variables, and the 31 different C substrates were used as explanatory variables in a canonical discriminant analysis (CDA). CDA provides a number of discriminant functions (CDFs) equal to the smaller of either the number of independent variables or the number of class variables minus one. Accordingly, two CDFs were generated considering the three PCB dissipation classes. These three classes were separated by including only 17 out of a total of 31 C substrates. In other words, utilization patterns of 17 carbon substrates on the Biolog plate discriminated among rhizosphere microbial populations under our various treatments. Table 2 shows that only CDF 1 discriminated among the three classes of PCB dissipation significantly (p<0.0001). A plot of the scores of CDF 1 and CDF 2 (Figure 1) provides a graphical representation of the discriminatory power of the two CDFs in separating PCB dissipation classes along the high, medium, and low classes.

Total canonical structures (TOC) whose absolute values indicate correlation coefficients between individual substrates, and the scores of each function are shown in Table 3. Total canonical structure can be used to infer the importance of each C substrate in the construction of each CDF. The substrates that were found to be important in defining CDF 1 cover the full range of substrate categories provided on the Biolog Ecoplate, namely, carboxylic acids, carbohydrates, polymers, amino acids, amines, and miscellaneous substrates. Interestingly, 12 out of the 17 substrates that seem important in defining CDF1 have not been reported as constituents of rhizosphere exudates (Campbell et al., 1997).
TABLE 1. PCB dissipation patterns under various treatments

<table>
<thead>
<tr>
<th>% of initial 50mg/kg PCB recovered</th>
<th>Treatment</th>
<th>Dissipation class</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.9 ± 4.3</td>
<td>All amendment treatments except biphenyl-burr medic combinations</td>
<td>High</td>
</tr>
<tr>
<td>62.4 ± 6.2</td>
<td>All unamended soils</td>
<td>Medium</td>
</tr>
<tr>
<td>84.2 ± 5.3</td>
<td>Biphenyl-amended, burr medic planted</td>
<td>Low</td>
</tr>
</tbody>
</table>

1 Means of three replicates

IV. DISCUSSION

Reed canarygrass and flat pea were selected for these studies based on their stimulation of PCB dissipation in a greenhouse study that was performed in Adelphi silt loam soil (Dzantor et al., 2000). The fact that the plants did not produce similar, significant stimulation of PCB dissipation in Hatboro silt loam soil used in these studies was not surprising; rhizosphere processes are strongly modulated by soil type (Buyer and Kaufmann, 1996; Buyer et al., 1999). Furthermore, the growth conditions of the two experiments were different. Still, our observations appeared to suggest a small trend toward stimulation of PCB dissipation under Hatboro rhizosphere soils compared with nonrhizosphere soil (e.g., nearly 70% of an initial 50 mg/kg addition of aroclor 1248 recovered in unplanted soil vs. 55% in burr medic-planted soil). The possibility that the generally lower recoveries of PCB in rhizosphere soil could be due to processes such as sorption of the chemicals onto roots was excluded by extracting macerated and thoroughly mixed samples of soil and roots. Lipophilic compounds like PCBs are taken up strongly by plant roots, but translocation of such compounds within plants is thought to be quite limited, if at all (Bromilow and Chamberlain, 1995). However, evidence, some dating back nearly 40 years, has shown that even weathered forms of some compounds in the same range of lipophilicity as PCBs are translocated in specific plant species. The phenomenon has been most widely reported in plant species belonging to the family Curcubitaceae (Lichtenstein...
TABLE 2. Canonical discriminant functions (CDFs) and their canonical correlations for the CDA

<table>
<thead>
<tr>
<th>CDF</th>
<th>Canonical Correlation</th>
<th>Eigenvalue (proportion, cumulative)</th>
<th>Approximate F value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.90</td>
<td>6.11</td>
<td>4.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.85, 0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.71</td>
<td>1.05</td>
<td>1.98</td>
<td>0.0515</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.15, 1.00)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1. Separation of PCB dissipation classes by two canonical discriminant functions (CDFs) using 17 carbon substrate variables on BIOLOG Ecoplates; ◊ Burr medic, biphenyl, □ Unamended, △ Amended.
### TABLE 3. Total canonical structure (TOC) for the two canonical discriminant functions

<table>
<thead>
<tr>
<th>Carbon Source Variable(^1)</th>
<th>Total Canonical Structure</th>
<th>Substrate category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDF1</td>
<td>CDF2</td>
</tr>
<tr>
<td>D-Glucosaminic acid</td>
<td>-0.580</td>
<td>0.450</td>
</tr>
<tr>
<td>1-Erythritol</td>
<td>0.425</td>
<td>0.452</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid*</td>
<td>-0.401</td>
<td>0.371</td>
</tr>
<tr>
<td>Glucogen</td>
<td>-0.302</td>
<td>0.413</td>
</tr>
<tr>
<td>a-Ketobutyric acid</td>
<td>-0.297</td>
<td>0.378</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.264</td>
<td>0.672</td>
</tr>
<tr>
<td>D-Cellobiose</td>
<td>-0.239</td>
<td>0.589</td>
</tr>
<tr>
<td>D-Xylose*</td>
<td>-0.255</td>
<td>0.553</td>
</tr>
<tr>
<td>a-Cyclodextrin</td>
<td>-0.187</td>
<td>0.353</td>
</tr>
<tr>
<td>D, L-a-Glycerol phosphate (?)</td>
<td>-0.176</td>
<td>0.354</td>
</tr>
<tr>
<td>L-Arginine*</td>
<td>-0.159</td>
<td>0.633</td>
</tr>
<tr>
<td>D-Galactonic acid y-lactone</td>
<td>-0.100</td>
<td>0.635</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>0.093</td>
<td>0.258</td>
</tr>
<tr>
<td>Putrescine</td>
<td>-0.079</td>
<td>0.552</td>
</tr>
<tr>
<td>Itaconic acid</td>
<td>0.076</td>
<td>0.417</td>
</tr>
<tr>
<td>2-Hydroxybenzoic acid*</td>
<td>0.022</td>
<td>0.121</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>-0.016</td>
<td>0.407</td>
</tr>
</tbody>
</table>

\(^1\) Only substrates marked with asterisks have been reported as constituents of root exudates.
It has been speculated that this capability may be related to production of substances that can increase the availability of lipophilic contaminants in soil as well as facilitate their translocation within plants (Hulster et al., 1994).

Uptake of PCB from soil and subsequent translocation has been reported for a limited number of crops, including tall fescue and soybeans (Weber and Mrozek, 1979; Pal et al., 1980). However, the reported amounts translocated were generally low, usually <0.5% of soil concentrations. It is most likely that in our experiments, some of the less lipophilic components of aroclor 1248 were translocated into the plants’ upper portions. Accordingly, there is a need to analyze plant tissue to determine the exact distribution of aroclor 1248 in these treatments. Still, comparison of data from amended, unplanted soils (average dissipation of 51% initial in 100 d) and unamended planted soils (average dissipation 40%) (Dzantor and Woolston, 2001) strongly suggest soil processes (most likely microbial and/or plant-microbe interactions) as the cause of aroclor 1248 dissipation, rather than contributions by plant uptake and translocation (PCB dissipation in unamended, unplanted soil averaged 31% of initial application). Our goal is to enhance processes that lead to contaminant destruction in soil over those involving translocation and metabolism in plant upper portions, which could still carry potential risks from accumulations of hazardous levels of parent contaminants and/or their transformation products.

Enhanced PCB dissipation in unplanted soil amended with biphenyl or the plant residues, orange peels, or pine needles was consistent with previous reports concerning enzyme induction for PCB biodegradation by similar amendments (Brunner et al., 1985; Hernandez et al., 1995). We hypothesized that the supply of appropriate inducers to already well-established competent PCB degraders in the selected plant rhizospheres could dramatically enhance the dissipation of the contaminant. Results from the soil amendment-planting treatment combinations did not show evidence of a synergy between the treatments. One explanation for the less than spectacular enhancement of PCB dissipation could be that while selected members of microbial communities might have been induced for PCB degradation, our treatments did not provide any significant boost to the size of the induced populations. In fact, plate counts showed that with the exception of depressed numbers in biphenyl-amended, burr medic-planted soil, there were generally no significant differences in cultivable bacterial populations among the various treatments (Dzantor and Woolston, 2001).

The possibility that selected members of populations within our treatments were induced for PCB degradation, yet remained undetected by plate enumeration approaches, underscores the need for functional analyses of communities, such as that afforded by the Biolog assay. The Biolog assay used during this study was quite limited in scope. For example, our measurements were made only on harvest time samples, which eliminated an evaluation of the impacts of our treatment on development of microbial communities over time. Still, the assays allowed us to differentiate among microbial communities under various soil amendments and planting treatment combinations, and to relate the community differences to differences in PCB dissipation under those treatments. Analysis of that information offered an insight into the relative importance of individual C substrates in differentiating among the microbial populations. Interestingly, the overwhelming majority of the substrates
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that were important in establishing these differences were compounds not usually included in listings of root exudates. The exact implications of this particular observation cannot be addressed from this study. However, if such patterns are confirmed through more extensive testing of different samples, then the BIOLOG assay can provide an additional tool for predicting rhizodegradation potentials of specific contaminants in particular matrices. Furthermore, the establishment of links between specific substrates and degrader populations for specific contaminants may suggest means by which sizes of competent populations might be selectively increased. This in turn will be invaluable in developing rhizodegradation for use in the field.

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REFERENCES


Dzantor et al.


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